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Phytochemical characterization and in-vitro remineralization potential of Citrullus lanatus seed aqueous extract on human enamel

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ABSTRACT

Objectives: To characterize the phytochemical composition of Citrullus lanatus seed aqueous extract and evaluate its remineralizing effect on demineralized enamel using surface microhardness testing supported by scanning electron microscopy (SEM). Methods: Characterization of an aqueous extract of C. lanatus seeds was done using FTIR, FAAS, UV–VIS, and GC–MS analyses. After preparation of fifteen sound human premolars, teeth were subjected to artificial demineralization, then randomly allocated to five groups: three concentrations of C. lanatus extract (1800, 2000, and 2200 mg/L), 5% sodium fluoride, and artificial saliva. Measurement of enamel surface microhardness was recorded at three stages: baseline, after demineralization, and after 14 days of treatment. To qualitatively assess surface morphology and mineral deposition, examination of representative specimens (sound enamel, demineralized enamel, artificial saliva, sodium fluoride, and C. lanatus extract groups) using SEM was utilized. Data were analyzed using one-way ANOVA and Tukey's post hoc test ($\alpha = 0.05$). Results: Analysis using FTIR and GC–MS resulted in identification of hydroxyl, carbonyl, and amide functional groups, with linoleic acid as the predominant organic constituent. Presence of magnesium, potassium and calcium ions was confirmed by FAAS. Significant increase in enamel microhardness was noticed following treatment with C. lanatus extract in 2000 and 2200 mg/L compared with artificial saliva ($p < 0.001$), with values comparable to sodium fluoride. These findings were corroborated by SEM observations, where the extract-treated enamel demonstrating surface porosity reduction and surface mineral deposition compared with demineralized and artificial saliva–treated specimens, with morphology comparable to sodium fluoride. Significance: Promising potential of enamel remineralization was demonstrated using Citrullus lanatus seed aqueous extract. This was supported by the recovery in the microhardness and SEM evidence. Under in-vitro conditions, the extract represents a plant-based biomaterial that exhibits remineralization performance within a comparable range to sodium fluoride.

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KEYWORDS
Citrullus lanatus; eco-friendly dentistry; enamel remineralization; plant-based biomaterials; microhardness

1. Introduction

Dental caries is considered a chronic and multifactorial disease caused by the progressive demineralization of enamel promoted by organic acids produced during bacterial fermentation of carbohydrates (1). It affects enamel microstructure, diminishes its surface microhardness, and could develop into cavitation if left untreated. Fluoride is considered the most important preventive agent against caries because of its capability for fluorapatite formation and improving resistance against acid attack. However, the use of non-toxic, biocompatible, and ecologically harmless alternatives is receiving increased interest inspired in part by concerns over systemic toxicity from excessive intake, dental fluorosis, and ecological contamination (2-4). As one of these, plant-derived biomaterials appear as promising candidates for bioactive remineralization of enamel.The extracts obtained from a variety of plant sources contain flavonoids, minerals, antioxidants, and polyphenols. These phytoconstituents may induce hydroxyapatite crystallization, augment calcium–phosphate deposition, thus stabilizing the organic matrix of enamel and maintaining its surface hardness (5-8). Such natural non-fluoridated remineralizing agents could avoid many disadvantages that are observed with traditional methods of remineralization (5).

Citrullus lanatus seeds have been of significant scholarly interest due to their high phytochemical composition. Empirical studies show that watermelon seeds are composed of calcium, magnesium, potassium, linolenic acid, phenolic compounds, and proteins that may serve in the replacement of mineral ions and in hydroxyapatite crystal growth in demineralized enamel (9–11). An extract is known to possess high antioxidant activity, which could neutralize oxidative reactions in the mouth and be useful in enamel stabilization.12 Due to its

biochemical nature, Citrullus lanatus seed extract is nontoxic and thus can be a useful alternative biomaterial in preventive dentistry.13-

While highly promising, the phytochemical profile of Citrullus lanatus seed extract has not yet been assessed for its efficacy in enamel remineralization and the restoration of microhardness. As a result, this is still a relatively underexplored area that assumes particular relevance in light of the present global rise in demand for effective fluoride-free remineralization strategies. This research has further reinforced the emphasis on green and environmentally friendly materials in modern preventive dentistry, calling for more research into biomaterials of plant origin for enamel repair.

Therefore, the present study aimed to (1) characterize the phytochemical composition of aqueous C. lanatus seed extract and (2) evaluate its effect on the remineralization of demineralized enamel surfaces by assessing changes in surface microhardness at different concentrations, in comparison with sodium fluoride and artificial saliva supported by scanning electron microscopy (SEM).

Hypotheses

• Null Hypothesis (H_0): Application of aqueous Citrullus lanatus seed extract at different concentrations produces no significant improvement in enamel remineralization or surface microhardness compared with sodium fluoride or artificial saliva.

• Alternative Hypothesis (H_1): Certain concentrations of Citrullus lanatus seed extract significantly enhance enamel remineralization compared with artificial saliva and demonstrate remineralization performance comparable to sodium fluoride.

2. Materials and Methods

2.1 Study Design

This in vitro comparative study was conducted at the University of Baghdad, College of Dentistry, and approved by the institutional ethics committee (Ref. 969/2024).

2.2 Sample Preparation

Fifteen human premolars, free of caries, cracks, or restorations, were extracted, cleaned, sectioned, and embedded in self-cure acrylic resin blocks. Sample size was based on previous in vitro remineralization studies and was limited by the availability of teeth from ethical sources. The buccal enamel surfaces were planarized and polished sequentially using silicon carbide papers (600–1200 grit) under water cooling. Specimens were stored at 4 °C in a 0.1% thymol solution until use(19-21).

2.3 Experimental Grouping

Group	Treatment	Concentration
G1	*Citrullus lanatus* seed extract	1800 mg/L
G2	*Citrullus lanatus* seed extract	2000 mg/L
G3	*Citrullus lanatus* seed extract	2200 mg/L
G4	Sodium fluoride (positive control)	5%
G5	Artificial saliva (negative control)	–

2.4 Extract Preparation

The seeds of Citrullus lanatus were washed, dried, and milled into fine powder. The powder was subjected to cold maceration in distilled water for 24 hours with occasional stirring(22) and was filtered and spray dried to produce an aqueous extract. Stock and working solutions (1800-2200 mg/L) were freshly prepared in distilled water(23).

2.5 Phytochemical Characterization

• FTIR spectroscopy (400–4000 cm^{-1}): Functional group identification.
• FAAS (Flame Atomic Absorption Spectroscopy): Quantification of calcium, magnesium, potassium, sodium, zinc, and iron.
• UV–VIS spectrophotometry: Determination of phosphate concentration.
• GC–MS (Gas Chromatography–Mass Spectrometry): Identification of major organic and bioactive compounds.

2.6 Enamel Microhardness Testing

Baseline enamel surface microhardness was measured with a Vickers microhardness tester for 15 seconds with a load of 200 g(19,20). The specimens were then subjected to 3 days of demineralization (pH 4.5, lactic acid buffer), followed by a 14-day treatment period of daily immersion at 37 °C in their respective solutions. Post-treatment microhardness values were measured at the same locations, and mean Vickers Hardness Numbers (VHN) were calculated for each group.

2.7 Scanning Electron Microscopy (SEM) Analysis

The SEM analysis was qualitative and intended to support microhardness findings rather than provide quantitative mineral analysis. For SEM analysis, one representative sample was prepared for each experimental group: sound enamel (control), demineralized enamel, enamel treated with Citrullus lanatus seed extract at 2000 mg/L, enamel treated with artificial saliva, and enamel treated with sodium fluoride. After microhardness testing, samples were washed with distilled water, air-dried, and then fixed on aluminum stubs using conductive carbon tape. All the samples were sputter-coated with a thin gold layer to improve surface conductivity(23). SEM observation was made in high-vacuum mode at an accelerating voltage of 15 kV. Micrographs were taken at standard magnification of ×5000 and ×10000 to observe enamel surface morphology, integrity of enamel prisms, porosity, and mineral deposition.

2.8 Statistical Analysis

The data analysis was performed by using the software IBM SPSS Statistics version 26. Tests for the normality of distributions were performed using the Shapiro-Wilk test. The mean VHNs among the groups were compared using one-way ANOVA and followed by Tukey's HSD post hoc test to solve the multiple comparisons problem. The significant probability level was defined as less than 0.05.

3. Results

3.1 FTIR Analysis

The FTIR spectrum of the aqueous Citrullus lanatus seed extract exhibited multiple absorption peaks confirming the presence of diverse functional groups (Table 1).

A broad band at 3280.54 cm^{-1} corresponded to O–H stretching vibrations, characteristic of hydroxyl groups from alcohols, phenols, or water molecules. Peaks at 3000.88, 2926.91, and 2861.77 cm^{-1} represented C–H stretching vibrations typical of aliphatic chains (alkanes).

Distinct peaks at 1743.71 cm^{-1} and 1635.69 cm^{-1} were attributed to C=O stretching of carbonyl and amide functional groups, respectively, indicating the presence of esters and proteins. Peaks at 1453.76 cm^{-1} and 1404.84 cm^{-1}, attributed to C–H bending or carboxylate (COO^{-}) vibrations. While that at 1236.19 cm^{-1} corresponding to C–O–C stretching (ethers); whereas those between 1154.13 cm^{-1} and 1085.16 cm^{-1}, representing C–O or P–O stretching vibrations of phosphate or carbohydrate compounds. Collectively, these bands confirmed that the extract contained a mixture of lipids, proteins, polyphenols, and phosphate-bearing constituents.

Table 1- FTIR peak interpretation of aqueous Citrullus lanatus seed extract.

Wavenumber (cm^{-1})	Functional Group	Possible Compound
3280.54	O–H Stretch	Phenols or Alcohols
3000.88	O–H or C–H Stretch	Alcohols or Alkanes
2926.91	C–H Stretch	Alkanes
2861.77	C–H Stretch	Alkanes
1743.71	C=O Stretch	Carboxylic Acids or Esters
1635.69	C=O Stretch / Amide I	Proteins or Peptides
1537.90	N–H Bend / Amide II	Proteins or Amides

1453.76	C–H Bend or COO^-	Carboxylates or Alkanes
1404.84	COO^- Stretch	Carboxylates group
1236.19	C–O Stretch	Esters or Ethers
1154.13	C–O Stretch	Carbohydrates or Alcohols
1085.16	P–O or C–O Stretch	Phosphates or Polysaccharides

3.2 Elemental Composition (FAAS and UV–VIS)

Flame atomic absorption spectroscopy revealed that the aqueous seed extract contained substantial quantities of magnesium, zinc, iron, calcium, potassium and sodium (Table 2). Magnesium showed the highest concentration (136.1 mg/L), followed by potassium (162.56 mg/L) and Calcium (127.83 mg/L). UV–VIS spectrophotometry indicated the presence of soluble phosphate ions (0.911 mg/L), supporting the potential of the extract to provide key ions required for hydroxyapatite crystal regrowth.

Table 2- Elemental composition of C. lanatus extract by FAAS and UV–VIS.

Mg mg/L	Zn mg/L	Fe mg/L	Ca mg/L	K mg/L	Na mg/L	(PO4) mg/L
136.1	3.551	3.112	127.83	162.56	34.213	0.911

3.3 GC–MS Profiling

Gas chromatography–mass spectrometry (GC–MS) analysis identified seven major organic constituents within the aqueous extract (Figure 1; Table 3).

The major components identified included linolenic acid ethyl ester, hexadecanoic acid, octadecanoic acid, and phytosterol derivatives. Most of these compounds are antioxidants, antimicrobial, and biocompatible molecules. Long-chain fatty acids and polyphenolic structures indicated the possible role in enamel remineralization by modulating mineral deposition and stabilizing the surface.

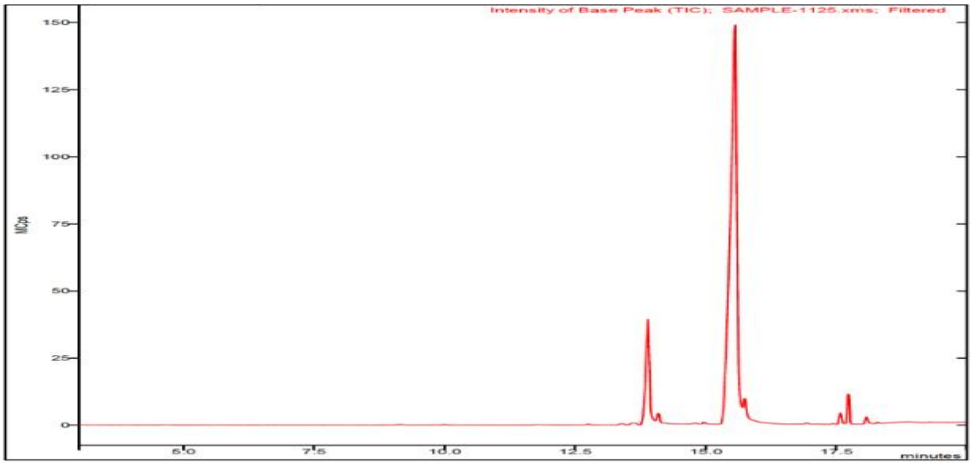


Figure 1- Total ion chromatogram (TIC) of aqueous Citrullus lanatus seed extract showing seven dominant peaks (14.08–18.08 min).

Table 3- Major organic compounds identified by GC–MS analysis of C. lanatus aqueous extract.

PEAK NUMBER	RETENTION TIME (MIN)	NAME	M.WT	PROB%	%TOTAL
PEAK 1	14.089	Undecanoic acid, ethyl ester	214	93	11.467

PEAK 2	15.469	9,12-Octadecadienoic acid (Z, Z)	280	67	0.143
PEAK 3	15.502	Linoleic acid	280	98	82.05
PEAK 4	15.746	3,10-Dioxatricyclo [4.3.1.0(2,4)] dec-7-ene	138	55	0.098
PEAK 5	17.58	cis, cis-7,10-Hexadecadienal	236	83	0.023
PEAK 6	17.742	Bis(2-ethylhexyl) phthalate†	390	90	0.044
PEAK 7	18.082	5,10-Pentadecadien-1-ol (Z,Z)	224	78	0.776

3.4 Enamel Microhardness

The results of the Vickers microhardness test are summarized in Tables 4 and 5. All specimens had a significant reduction in mean hardness from baseline values after three days of demineralization ($p < 0.001$). However, after treatment for fourteen days, significant restoration of surface microhardness occurred with Citrullus lanatus extract and sodium fluoride groups. Among the experimental groups, G3 (2200 mg/L) exhibited the highest mean hardness value (271.29 ± 5.17 HV), followed closely by G2 (2000 mg/L; 265.82 ± 5.86 HV). Both G2 (2000 mg/L) and G3 (2200 mg/L) exhibited significantly greater remineralization than artificial saliva ($p < 0.001$). Although the difference between all the extract groups and sodium fluoride was statistically significant ($p < 0.05$), the recovery amount in terms of microhardness remained within comparable magnitudes in vitro. In contrast, the artificial saliva group had the lowest recovery (150.91 ± 3.66). These findings suggested that treatment with watermelon seed extract, particularly with 2000–2200 mg/L, effectively enhanced enamel remineralization and restored microhardness values close to those of the intact enamel surface.

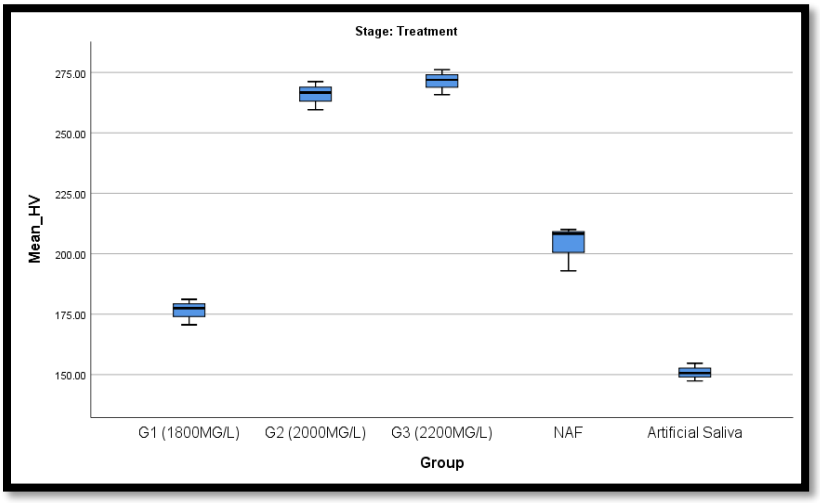


Figure 2- Boxplot illustrating Vickers microhardness (VHV) distribution across groups during the treatment stage.

Table 4- Descriptive statistics of mean ± SD Vickers microhardness (VHV) at each stage.

STAGE	GROUP	MEAN	SD	SE	MIN	MAX
BASELINE	G1 1800 mg/L	436.35	21.84	12.61	421.89	461.47
	G2 2000 mg/L	384.94	22.53	13.01	363.16	408.16
	G3 2200 mg/L	405.5	20.48	11.82	388.82	428.36
	NaF	397.07	13.61	7.86	387.77	412.7
	Artificial Saliva	308.65	12.17	7.03	297.46	321.61
DEMINERALIZATION	G1 1800 mg/L	105.03	1.58	0.91	103.87	106.83
	G2 2000 mg/L	126.68	9.75	5.63	117.9	137.18
	G3 2200 mg/L	122.98	2.45	1.41	121.09	125.74

	NaF	100.22	2.26	1.3	97.62	101.53
	Artificial Saliva	148.12	3.42	1.97	145.93	152.05
TREATMENT	G1 1800 mg/L	176.4	5.34	3.08	170.6	181.11
	G2 2000 mg/L	265.82	5.86	3.38	259.58	271.21
	G3 2200 mg/L	271.29	5.17	2.99	265.82	276.11
	NaF	203.72	9.41	5.43	192.91	210.01
	Artificial Saliva	150.91	3.66	2.11	147.39	154.7

Table 5- ANOVA and Tukey HSD results for inter-group comparisons of post-treatment microhardness.

STAGE	I GROUP	J GROUP	MEAN DIFF I-J	STD. ERROR	SIG. P
BASELINE	G1 (1800 mg/L)	G2 (2000 mg /L)	51.418*	15.22	0.044
	G1 (1800 mg/L)	G3 (2200 mg /L)	30.853	15.22	0.32
	G1 (1800 mg/L)	NaF	39.281	15.22	0.148
	G1 (1800 mg/L)	Artificial Saliva	127.701*	15.22	0
	G2 (2000 mg/L)	G3 (2200 mg /L)	-20.565	15.22	0.669
	G2 (2000 mg/L)	NaF	-12.138	15.22	0.926
	G2 (2000 mg/L)	Artificial Saliva	76.282*	15.22	0.004
	G3 (2200 mg/L)	NaF	8.427	15.22	0.979
	G3 (2200 mg/L)	Artificial Saliva	96.847*	15.22	0.001
	NaF	Artificial Saliva	88.420*	15.22	0.001
DEMINERALIZATION	G1 (1800 mg /L)	G2 (2000 mg /L)	-21.651*	4.01	0.002
	G1 (1800 mg /L)	G3 (2200 mg /L)	-17.948*	4.01	0.008
	G1 (1800 mg /L)	NaF	4.805	4.01	0.752
	G1 (1800 mg /L)	Artificial Saliva	-43.088*	4.01	0
	G2 (2000 mg /L)	G3 (2200 mg /L)	3.704	4.01	0.881
	G2 (2000 mg /L)	NaF	26.456*	4.01	0
	G2 (2000 mg /L)	Artificial Saliva	-21.437*	4.01	0.002
	G3 (2200 mg /L)	NaF	22.752*	4.01	0.001
	G3 (2200 mg /L)	Artificial Saliva	-25.140*	4.01	0.001
	NaF	Artificial Saliva	-47.893*	4.01	0
TREATMENT	G1 (1800 mg /L)	G2 (2000 mg /L)	-89.422*	5.05	0
	G1 (1800 mg /L)	G3 (2200 mg /L)	-94.894*	5.05	0
	G1 (1800 mg /L)	NaF	-27.328*	5.05	0.002
	G1 (1800 mg /L)	Artificial Saliva	25.485*	5.05	0.004
	G2 (2000 mg /L)	G3 (2200 mg /L)	-5.472	5.05	0.811
	G2 (2000 mg /L)	NaF	62.094*	5.05	0
	G2 (2000 mg /L)	Artificial Saliva	114.907*	5.05	0

	G3 (2200 mg /L)	NaF	67.565*	5.05	0
	G3 (2200 mg /L)	Artificial Saliva	120.379*	5.05	0
	NaF	Artificial Saliva	-52.814*	5.05	0

3.5 Scanning Electron Microscopy (SEM) Analysis

Micrographs by SEM demonstrated distinct morphological differences among the examined enamel surfaces:

1-Sound Enamel

SEM micrographs of sound enamel surfaces show a somewhat compact surface featuring well-organized enamel prisms and a smooth, homogeneous morphology that includes low surface roughness. The minute surface irregularities imply an intact mineralized enamel structure (Fig. 3).

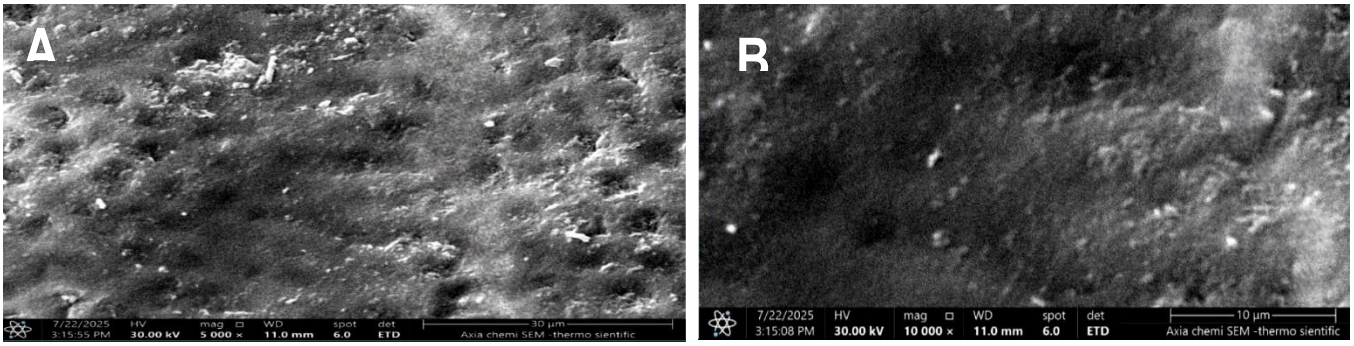


Figure 3. Scanning electron micrograph at different magnifications: (a) 5000× and (b) 10000×, showing a comparatively smooth and homogeneous surface morphology of sound enamel surfaces

2. Demineralized Enamel

Figure 4 displayed pronounced surface modulation of demineralized enamel compared with sound enamel by SEM images. A marked increase in surface roughness was detected, along with a characteristic honeycomb-like appearance and multiple porosities, suggesting substantial mineral loss. Depressed and irregular surface areas were additionally noted, reflecting the demineralization process subsequent to acid challenge.

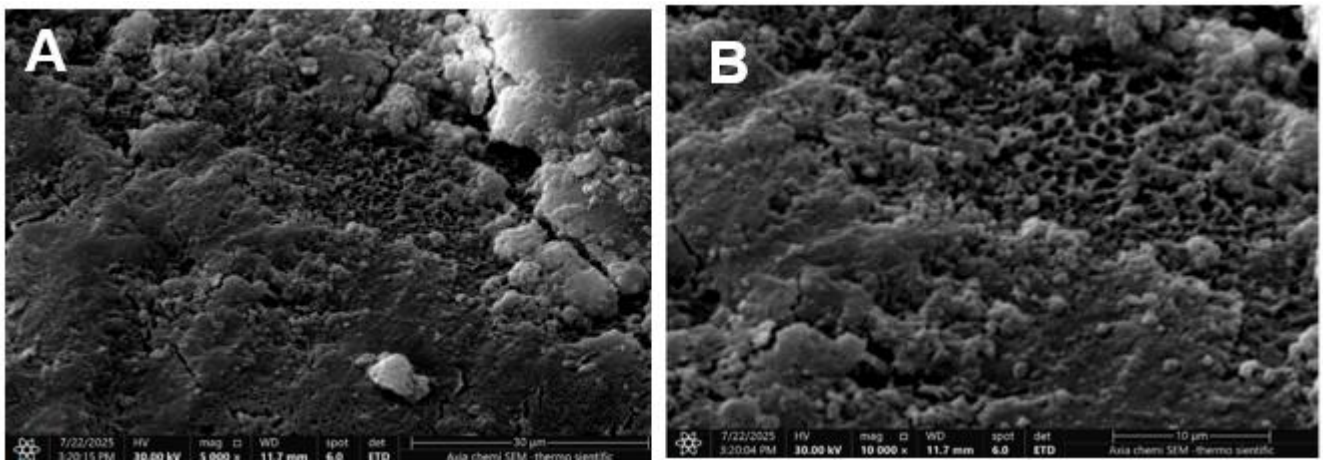


Figure 4. Scanning electron micrograph at different magnifications(a)5000× and (b) 10000×, of demineralized enamel revealing increased surface roughness and porous.

3. Enamel Sample Treated with Aqueous Watermelon Seed Extract

The SEM micrographs of the demineralized enamel sample treated with watermelon seed extract showed significant recovery of the surface. Overall, there was a reduction of surface porosity and partial occlusion of areas previously subjected to demineralization. There were mineral-like irregular deposits on the surface of the enamel, and the surface is more compact and continuous. This suggests

surface mineral deposition associated with changes in remineralization, as depicted in Fig. 5.

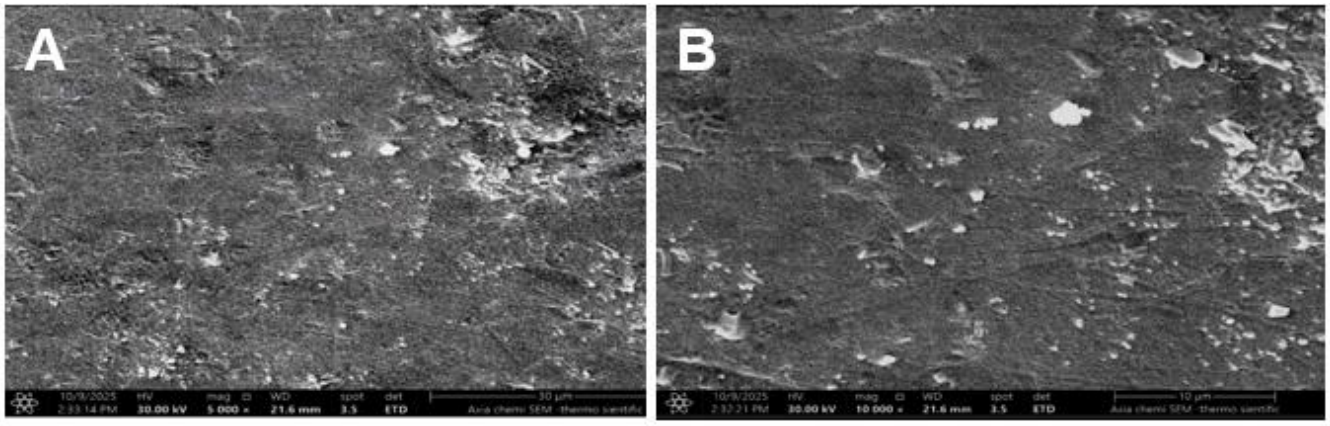


Figure 5. Scanning electron micrograph at different magnifications; (a) 5000× and (b) 10000×, of demineralized enamel treated with watermelon seed extract showing partial occlusion of porosities and mineral-like precipitation.

4. Enamel Treated with Sodium Fluoride

4. Enamel sample Treated with Sodium Fluoride 5%

Figure 6 showed SEM Images of enamel sample treated with 5% NaF that revealed surface smoothing and mineral precipitation when compared with demineralized enamel. Although a degree of surface remineralization was clear, some surface irregularities remained. These findings demonstrate the well-documented role of fluoride in enhancing partial enamel remineralization, Fig. 6.

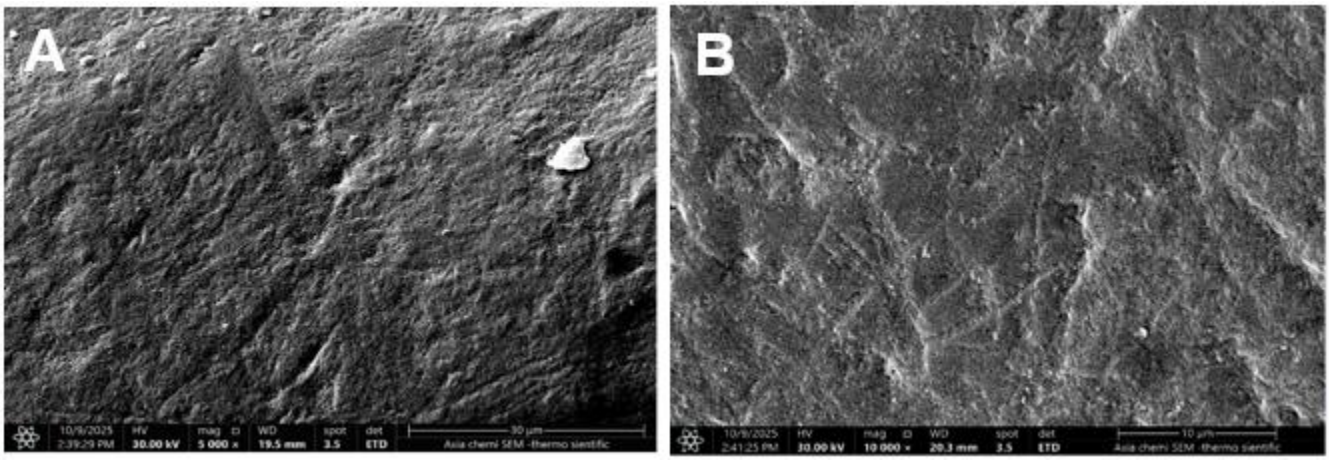


Figure 6. SEM micrographs of demineralized enamel at different magnifications: (a) 5000× and (b) 10000×, treated with fluoride demonstrating surface smoothing and mineral deposition.

5. Enamel sample Treated with Artificial Saliva

SEM observations of the enamel specimen treated with artificial saliva showed partial recovery of the surface. The surface roughness and porosities were still present in most regions, with sparse and non-homogeneous mineral deposition. All these facts suggest that artificial saliva gives a negligible remineralization effect compared to the Watermelon Seed Extract and Fluuoride treated samples, as shown in Fig. 7.

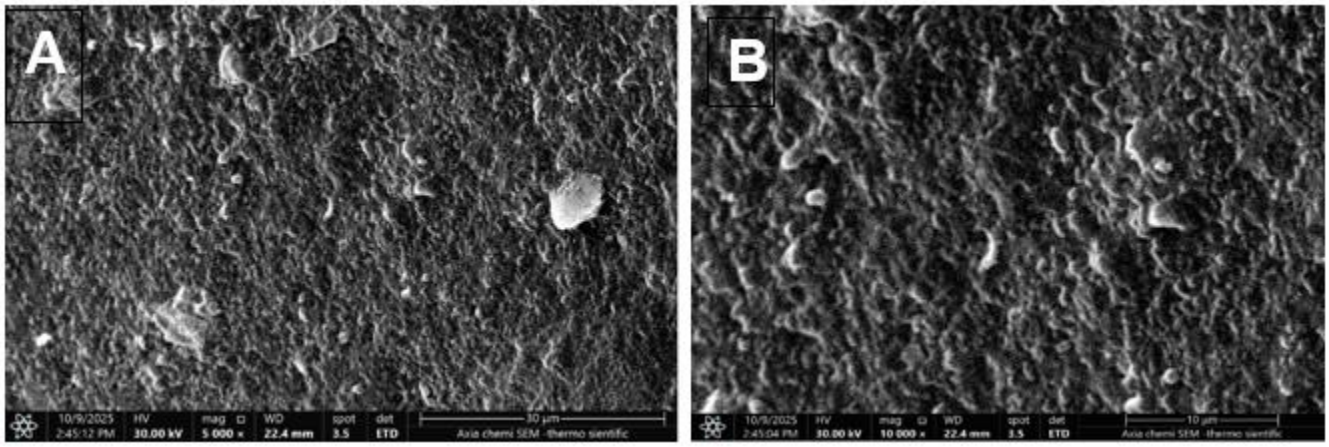


Figure 7. Scanning electron micrograph at different magnifications: (a) 5000× and (b) 10000×, of demineralized enamel treated with artificial saliva indicating limited surface remineralization

3.6 Summary of Findings

The above results suggest that the aqueous extract of Citrullus lanatus seeds is rich in a broad spectrum of bioactive compounds and inorganic ions that might enhance enamel remineralization. At both 2000 mg/L and 2200 mg/L, increases in enamel surface hardness were statistically significant for the extract and similar to the hardness in enamel treated with 5% sodium fluoride. In general, scanning electron microscopy observations revealed that the C. lanatus seed extract increased mineral deposition and maintained more enamel surface integrity compared with both demineralized enamel and enamel treated with artificial saliva. Thus, C. lanatus seed extract is a promising biomaterial derived from plants with possible advantages in biocompatibility for natural restoration of enamel.

4. Discussion

Analysis by FTIR confirmed the presence of functional groups known to be a contributing factors in biological activity and surface affinity these groups are alcohols, , fatty acids, phenolics, esters and amides molecules, The presence of hydroxyl (–OH), carbonyl (C=O), and amide bands aligns with findings from Bichi (14), who also reported similar spectral patterns in Citrullus lanatus extracts, attributing them to antioxidant and chelating agents that may promote mineral binding and remineralization. Comparable functional groups have also been reported in other plant extract including Nigella sativa extracts and green tea derivatives (16,25-27), suggesting a conserved biochemical pattern among plant-based materials with promising enamel-protective benefits.

The result that supports the promising capacity of watermelon seed extract in mineral replacement of demineralized enamel was its high mineral content, particularly magnesium, calcium , and potassium (28-31), A comparable remineralizing potential has been reported for grape seed extract, where the proanthocyanidin-rich composition enhanced enamel microhardness by promoting calcium and phosphate deposition on demineralized enamel surfaces, forming spherical Ca–P complexes observed under SEM-EDX analysis (32). The current study provides supporting indicator that C. lanatus extract may act via a similar mechanism, supplemented by its mineral and organic matrix constituent.

The predominance of unsaturated fatty acids in the extract is consistent with the high unsaturated-fat content of watermelon seeds reported by Nissar et al. (33), which associated these lipids with antioxidant potential. With their amphiphilic character, they probably could enhance the surface adhesion and stabilize mineral deposition, despite the direct evidence for their involvement in enamel protection remaining scant. Indeed, previous studies have documented the antimicrobial properties of Citrullus lanatus seed extracts, whose active constituents were identified as fatty acids, phenolics, and coumarin-type compounds (34) that could provide additional ancillary benefits against cariogenic bacteria. All the mean baseline microhardness values obtained in this study were higher than the values normally mentioned for unpolished enamel surfaces. This result confirms previous reports that baseline hardness is higher in polished or enamel-flat surfaces(35-39). The polishing process removes surface irregularities and the outer prismatic layer, leaving a more homogenous and densely mineralized surface, which can affect the measurement of subsequent remineralization. Featherstone and ten Cate (35) said that enamel preparation methods alter the baseline hardness. Similarly, Hicks et al.(36) and Vashisth et al.(37) used polished enamel samples in reporting comparable findings. Rirattanapong and Saengsiri(38) and Hara and Zero (39) further confirmed that the initial microhardness is higher for smooth, flat enamel surfaces. These facts must be borne in mind when interpreting the results of remineralization.

The current study shows that an aqueous extract of Citrullus lanatus seeds strongly enhances the remineralization of artificially

demineralized enamel. The increase in microhardness reached its maximum at extract concentrations of 2000 mg/L and 2200 mg/L. This effect can be explained by a synergistic interaction between the mineral constituents and organic compounds within the extract, which have been characterized by FTIR, FAAS, and GC–MS. The observed increase in microhardness was equivalent to the positive effect of a 5% sodium fluoride solution and thus this seed extract might act as a promising plant-based remineralizing agent in vitro.

These findings are in agreement with Sultan (40), who showed that pastes containing chia and flaxseed significantly increased enamel microhardness and induced calcium–phosphate deposition to an extent similar to fluoride varnish. Taken together, results are consistent with an increasing number of studies demonstrating the potential of mineral, fatty acid, and polyphenol-rich plant materials, specifically Citrullus lanatus seed extract, to restore surface integrity through ion replenishment and matrix stabilization and provide a chemical and biological mechanism of action for Citrullus lanatus-watermelon-seed extract. Hydroxyl and carbonyl functional groups are predicted to provide adsorption sites for calcium and phosphate ions on the demineralized enamel surface, which promotes nucleation and regrowth of hydroxyapatite crystals (41).

In addition, it is plausible that the esters of fatty acids, such as linolenic acid, act as surface-binding agents, creating a semi-organic matrix that stabilizes crystal nucleation, an effect similar to that of casein phosphopeptides (42, 43). Thus, a product containing both polyphenolics and minerals could provide a dual mechanism supportive of antioxidant protection and mineral replenishment.

(Although qualitative, the changes in the surface structure observed by SEM analysis supported the microhardness recovery in all cases and, therefore, functional remineralization findingsحسيته ركيك اذا بي مجال يتعدل).. This is direct morphological evidence for the remineralization potential of Citrullus lanatus seed extract. Artificial saliva was only able to accomplish partial recovery of the enamel surfaces, while the extract produced a more compact, mineralized enamel surface, characterized by decreased porosity and prism obliteration. These features were compatible with the microhardness test results, suggesting that the extract promotes surface mineral deposition and stabilization of the enamel. This work responds to the continuing interest in finding less toxic and more biocompatible fluoride alternatives that are less likely to be associated with fluorosis and/or ecological toxicity. It identifies C. lanatus seed extract as a natural biomaterial with remarkable potential for efficient and long-lasting restoration of enamel hardness. The comparable remineralization ability of the extract to sodium fluoride indeed warrants its development as a fluoride-free, adjunctive strategy for effective remineralization with in vivo and clinical validations, following the renewed interest in natural product-based interventions (46-50).

5. Conclusion

Collectively, all in vitro results obtained up to date on the remineralization activity of the aqueous extract of Citrullus lanatus seed on demineralized enamel are encouraging. Precisely, at concentrations of 2000 and 2200 mg/L, the extract significantly enhanced enamel microhardness, exceeding restoration obtained with artificial saliva and producing a remineralization effect akin to that produced by sodium fluoride.

The detection of in organic ions such as calcium, magnesium, potassium, and bioactive compounds as linolenic acid and phenolics likely played a role in facilitating mineral deposition and stabilizing the enamel matrix suggesting that C. lanatus seed extract acts through the dual action include mineral replenishment and organic matrix-mediated pathways to promote hydroxyapatite regrowth.

These findings that supported by microhardness recovery and SEM observations suggest that the aqueous extract of Citrullus lanatus (watermelon) seeds has potential as a plant-based biomaterial for further development as a non-fluoride remineralizing strategy. Further in-vivo and clinical studies are needed to confirm its long-term efficacy and safety.

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Conflict of Interest

The authors declare no conflicts of interest.

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